RESEARCH

Emission Rates, Survival, and Modeled Dispersal of Viable Pollen of Creeping Bentgrass

W. Pfender,* R. Graw, W. Bradley, M. Carney, and L. Maxwell

ABSTRACT

Dispersal and deposition of pollen of creeping bentgrass (*Agrostis stolonifera* L.) was estimated by using CALPUFF, a complex model originally developed to simulate dispersal of particulates and other air pollutants. In field experiments, peak pollen emission rates (8×10^6 pollen grains per min per m^2 of a creeping bentgrass stand) occurred between 1000 and 1200 h. Pollen survival under outdoor conditions decreased exponentially with time, and only 1% survived for 2 h. CALPUFF simulations showed deposition of 100,000 viable pollen grains per m^2 at distances of 2 to 3 km from the source field, and deposition of one pollen grain per 10 m^2 at distances of 4.6 to 6.7 km from the source field. Pattern of simulated deposition varied with weather conditions and, to a lesser extent, source field size. Simulation of dispersal by a small thermal vortex produced deposition of one grain per 10 m^2 at 15.3 km from the source field. Overall, the deposition modeling results suggest that pollen-mediated gene flow is likely at distances of 2 to 3 km from a source field, and possible at distances up to 15 km.

W. Pfender and M. Carney, USDA-ARS National Forage Seed Production Research Center, 3450 SW Campus Way, Corvallis, OR 97331; R. Graw, USDA Forest Service Pacific Northwest Region, P.O. Box 3623, Portland, OR 97208; W. Bradley and L. Maxwell, Dep. Botany and Plant Pathology, Oregon State Univ., Corvallis, OR 97331. Received 19 Jan. 2007. *Corresponding author (pfenderw@onid.orst.edu).

Abbreviations: GMO, genetically modified.

The issue of pollen-mediated gene flow has become increasingly important in recent years with proposed and actual introduction of genetically modified crop plants into agriculture (Johnson and Riordan, 1999; Rieger et al., 2002; Watrud et al., 2004). A major concern is movement of transgenes from the genetically modified (GMO) crop into cultivated or native plants of the same or closely-related species (Hanson et al., 2005; Messeguer et al., 2004; Schmidt and Bothma, 2006). The potential for such gene flow is especially great in grasses, because of their wind-dispersed pollen and the wide distribution and adaptation of many grass species (Rognli et al., 2000). Information on pollen movement from a grass field, including an approach to estimate the movement under a variety of conditions, would therefore be useful.

Several studies report observations of effective pollination distances for grasses. Using a small area of source plants, with recipient plants placed at various distances from the source, investigators have used progeny analysis to detect transfer of identifiable genes from source to recipient plants. By this approach, pollen of tall fescue (*Festuca arundinacea* Schreb.) was found to effect fertilization of plants at distances of at least 325 m (Nurminiemi et al., 1998) and 330 m (Rognli et al., 2000) from the source (the

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677 S. Segoe Rd., Madison, WI 53711 USA

largest distances tested in each case). In contrast, another study using a relatively small number of source plants detected pollination at a distance of 150 m, but not 200 m (Wang et al., 2004). An experiment with bentgrass (*Agrostis* spp.) produced observations of pollination at distances of approximately 300 m (0.02% of the progeny positive for the marker gene) (Wipff and Fricker, 2001). Fitting a simple exponential decline with distance to these latter data suggests recipient plants at 1.3 km from the 286 source plants could have been fertilized. In another experiment, nontransformed *Agrostis* spp. plants, naturally occurring or intentionally placed as sentinels, produced transgenic seed after being exposed up to 14 to 21 km from a 162-ha source of GMO creeping bentgrass (*A. stolonifera* L.) in Oregon (Watrud et al., 2004).

Attempts have been made to generalize or model effective dispersal distances for wind-blown pollen. Some pollination distance models have been concerned only with short-distance data, a maximum of 15 m (Belanger et al., 2003; Meagher et al., 2003), but others have attempted to describe longer-distance phenomena. Rognli et al. (2000) noted that pollen-mediated gene flow depends on dispersal distance of pollen grains, their viability, and the environment, and on the presence of competing pollen at the recipient plants. All published reports have shown that pollination decreases with distance from the source plants, generally as some form of a negative exponential function displaying leptokurtosis (higher probability distributions in the tails than predicted by a normal distribution) (Gleaves, 1973; Rognli et al., 2000).

Giddings et al. (1997a) attempted to model pollen dispersal from a perennial ryegrass source to trap plants located at distances up to 80 m. He found that previously-reported equations for dispersal (i.e., negative exponential with a general factor to reflect wind turbulence) were not very useful to describe his observations. Adding factors for wind direction (Giddings et al., 1997b) did not improve results greatly, and the author noted that dispersal did not always decrease smoothly with distance and that multiple factors are likely needed to explain this complex phenomenon. Nurminiemi et al. (1998) fit several models, built on exponential decrease with distance, to data derived from a pollination experiment with marker genes in tall fescue. They selected a model that could predict the main patterns in the data (i.e., dispersal inversely proportional to distance and with distinct leptokurtosis, an effect of wind direction, and a strong effect of competing pollen at recipient location), but there were discrepancies at some of the greater distances (i.e., 160 m) tested. Rognli et al. (2000) likewise found it difficult to predict pollen distribution >155 m from the source, but could generally model pollination to be more than exponentially leptokurtic with distance, and to depend on source characteristics (i.e., size, distribution, and density) and wind direction. A review of the physical factors involved in pollen dispersal (Jackson and Lyford, 1999) notes that atmospheric instability has a major effect on dispersal distances. Adding to the complexity is the fact that pollen is likely emitted from a source field as a series of puffs, rather than as a steady Gaussian plume which would be simpler to characterize (Jackson and Lyford, 1999).

Dispersal modeling has been addressed in areas of biometeorology other than pollination. For example, dispersal of plant pathogenic fungal spores from infested fields has received much attention (Aylor, 1986, 1999). We recently published the use of a complex air pollution model (CALPUFF) to estimate dispersal of fungus spores from grass-seed fields infested with the stem rust fungus (Pfender et al., 2006). CALPUFF is an air pollution modeling system originally developed for estimating movement and deposition of air pollution contaminants, including particulates, for both short and long distances (Scire et al., 1990). It is in widespread use by air quality regulation agencies, and has been validated in several studies with controlled releases of tracer gases. In one study, where the tracer was released in a rural setting and detectors were arranged in 12 arcs ranging from 0.5 to 50 km from the source, mean concentrations of the tracer modeled by CALPUFF/CALMET were 98% of the actual, and the modeled maximum concentrations were 79% of actual (Hurley and Luhar, 2005). In a different study conducted in a region with desert basins and mountains and detectors up to 20 km from the source, 50 to 60% of the CALPUFF predictions were within a factor of two of observed tracer levels when the release was from a point source, and 25 to 30% were within a factor of two when the release was from line sources (Chang et al., 2003). CALPUFF has been used to model dispersion of particulates, also. It was able to reproduce the observed time series of 10-μm particulates recorded at surface monitors in a New Zealand study (Barna and Gimson, 2002). The CALPUFF modeling system allows the inclusion of such realistic elements as variations in wind speed, direction, and turbulence. It is a non-steady-state Lagrangian Gaussian puff model with modules for gridded, time-varying, three-dimensional meteorological conditions, complex terrain effects, and wet and dry deposition. Technical details are available in Scire et al. (1990), and a narrative summary of its salient features was presented in Pfender et al. (2006). The meteorological pre-processor, CALMET, uses prognostic output from the Penn State Mesoscale Meteorological Model (MM5) as an initial estimate for the windfield, which is then modified to account for effects of complex terrain (Scire et al., 2000). The results are interpolated to 2.5 km resolution, adjusted based on surface and upper-air observations, and used as input for CALPUFF. CALPUFF also allows the use of local weather observations, including wind turbulence measurements, obtained at the release site. In addition to weather information CALPUFF uses inputs for particle (pollen) size and settling velocity, the source field size and the pollen emission rate for each time unit of the modeled period. The model tracks the mass of particles emitted from the source, the amount deposited at any selected receptor sites in the modeling domain, and the amount remaining in the atmosphere (surface mixed layer and the air above the mixed layer) for each model time interval. The inclusion of both deposition (Pleim et al., 1984) and dispersion algorithms in CALPUFF, combined with the three-dimensional meteorological and land-use field, should result in more accurate model-predicted results compared with simpler models based on a steady-state Gaussian plume description.

In addition to information about dispersal distance of pollen, survival dynamics must be known to evaluate probability of pollination at various distances from the source (Luna et al., 2001). Grass pollen is relatively short-lived, typically <3 h (Huang et al., 2004; Teare et al., 1969). Fei and Nelson (2003) collected pollen of creeping bentgrass cultivar Crenshaw, stored it in a desiccator, and tested germination at 20-min intervals. Germination was approximately 80% during the first hour after shedding, then dropped to 20% at 80 min and to 0% by 140 min after shedding.

In this paper, we use CALPUFF to estimate dispersal distances and deposition rates of viable pollen from fields of creeping bentgrass. Additional supporting objectives were to estimate survival dynamics and the settling rate of bentgrass pollen grains, and to determine the rate of emission of pollen from flowering stands of bentgrass.

MATERIALS AND METHODS

Pollen Characteristics

Pollen was collected from plants of creeping bentgrass cultivar Seaside obtained from a field near Corvallis, OR. Flower heads were cut in the morning before flowers opened, the stems were placed in water and transported to the laboratory. Flower heads were placed on glassine paper and the pollen was collected as the anthers opened. Average weight per pollen grain was determined by weighing a several-milligram sample, then determining number of grains in the sample and dividing weight by number of grains. Number of grains was determined by suspending the weighed sample in a known volume of water, and counting the number of grains per 0.1 mm^3 with the use of a haemocytometer. The size of hydrated pollen was determined by measuring diameters of 50 pollen grains after mounting in a solution of glycerol/water (30:70) (Stanley and Linskens, 1974).

Settling velocity was measured with the use of a settling tower, as previously described (Pfender et al., 2006). A very small amount of pollen was released through a pinhole at the top of a glass tube (45 cm tall, 5 cm diam.), and collected on a series of greased microscope slides moved at 1-s intervals through a 3-mm high gap below the tube. Light directed upward through the tube from a cool fiber-optic source allowed us to observe that the pollen fell uniformly, without turbulence. Each slide was examined microscopically to count pollen grains that reached the bottom of the tube in that time interval. Average and variance of the settling rate for pollen grains were calculated based on the time required to fall the length of the settling tower. The experiment was conducted four times.

Pollen Survival Dynamics

Plants of cultivar Seaside were grown in pots outdoors. When anthers first emerged in the morning, the pots were taken into a greenhouse and observed continuously for anther dehiscence. Within 15 min of dehiscence, pollen was collected for testing.

To test pollen survival under conditions that would mimic those during aerial dispersal (exposure to ambient radiation and relative humidity in free air), yet permit us to recover the pollen for viability testing, we exposed pollen on traps made of bird feathers. Each trap consisted of a plastic pot label (9.5 by 2.2 by 0.1 cm, Hummert International, Earth City, MO) with a rectangular window (5.0 by 1.5 cm) cut out. Three individual downy barbs from down feathers of barn owl (*Tyto alba*) were arranged side-by-side 2 mm apart and glued to the pot label such that each feather spanned the 1.5-cm opening width and the hookless barbules of the adjacent feathers overlapped. Before use, the down feathers were washed with a 1:1 mixture of methanol and methylene dichloride, rinsed three times in water (the first rinse for 8 h) to remove any fatty acids and alcohols that might interfere with biological processes (Shawkey et al., 2003). Freshly-shed pollen was applied to each trap by holding it beneath creeping bentgrass flowers with newly-dehiscent anthers and tapping the flowers to release pollen.

Immediately after loading, each trap was taken outdoors and exposed in an unshaded location by clipping the plastic pot label frame to a rack. At 0, 10, 30, 60, 90, 120, and 180 min after beginning exposure, viability of pollen was tested on Petri plates of a germination medium described by Fei and Nelson (2003). Pollen was transferred by briefly pressing the feathers against the surface of the medium. Plates were incubated at 20 ± 2°C for 90 min, then pollen grains were examined microscopically to determine percent germination. A grain was considered germinated if the germ tube was at least two pollen diameters in length. The test was conducted on eight different days between 14 June and 5 July 2005. On each day of testing, there were three exposure traps per exposure time. Across the different days of testing, outdoor temperatures during exposure ranged from 17 to 24°C and greenhouse temperatures ranged from 22 to 27°C.

Field Experiments for Pollen Emission

Pollen emission rates (pollen grains emitted per m^2 field per min) and associated weather conditions were measured in field experiments conducted at Hyslop-Schmidt Experiment Farm (44° 38′ N, 123° 12′ W) near Corvallis, OR. Creeping bentgrass cultivar Seaside was established from transplants as a circular plot, 6 m in diameter, in a 1.2 ha field in January 2005. The remainder of the field surrounding the creeping bentgrass plot was planted to oats (*Avena sativa* L.). The oats were mowed during June and July as needed to maintain a canopy height similar to that of the creeping bentgrass. There was no other creeping bentgrass within 1 km of the study area, or within 2 km upwind on sampling days.

Pollen emitted from the creeping bentgrass plot by the action of naturally-occurring wind was measured with an array of samplers set in an arc downwind from the plot (Fig. 1), similar to the method previously reported for fungus spore emission measurements (Pfender et al., 2006). The arc was located at a radius of 10 m from the plot center, with samplers mounted on poles and set at intervals of 22.5° (approximately 3.8 m) along the arc. There were seven sampler poles (135° of arc), and the array was adjusted for each run to be centered on the downwind direction. The five middle poles of the array had samplers at heights of 0.5, 1.5, and 2.5 m. The center pole had an additional sampler at 4.0 m height. The two outer poles each had only a single sampler, at 0.5 m height (Fig. 1). Four additional samplers, to detect spores entering the study site from upwind, were deployed on the 10-m radius arc, centered 180° from the downwind center pole. The samplers were rotary impaction devices (Aerobiology Research Laboratories, Ottawa, ON K2E 7Y5) that collect airborne particles on square polystyrene rods (1.6 mm by 1.6 mm by 28 mm) coated on the leading edge with silicone grease. There are two rods per sampler, located at the ends of a 9-cm long arm that spins at 2400 rpm. The greased rods are retracted (protected from contamination) until the unit is switched on and again after it is switched off. For each sampling, the 22 samplers were turned on simultaneously and allowed to run for 15 or 30 min before being turned off.

The number of pollen grains per sampling rod was counted by examining with a microscope at 320× magnification. Because the samplers were located near the pollen source,

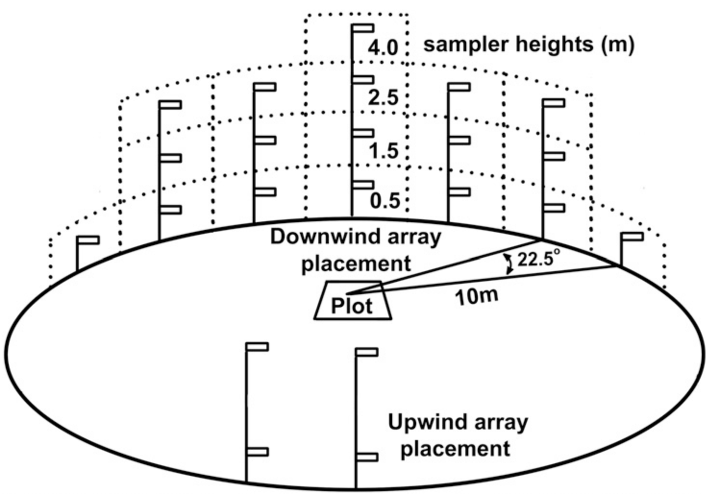

Figure 1. Sampling array for measuring number of pollen grains in a plume emitted from a plot of creeping bentgrass. Rotary impaction samplers were mounted on poles at heights of 0.5, 1.5, 2.5, and 4.0 m. The poles were set at 22.5° intervals along a 135° arc with a radius of 10 m, downwind from the center of a 6- by 6-m plot of creeping bentgrass. Two additional poles with samplers at 0.5 and 2.5 m height were placed upwind of the plot to measure background pollen concentrations. All samplers were run simultaneously during the sampling period of 15 or 30 min. To estimate total pollen passing through the downwind sampling array during the sampling period, the plume cross-section was divided conceptually into rectangles (indicated here in dashed lines). Pollen flux through each rectangle was derived by multiplying the spore concentration measured at the appropriate sampler by air volume moving through that rectangle. Air volume was calculated from measured or interpolated wind speed at sampler height, sampling duration, and cross-sectional area of the rectangle. The pollen fluxes of all rectangles were summed to produce the total flux.

and there was no other grass pollen source in the immediate vicinity, most pollen grains matched the size and morphology of creeping bentgrass pollen, but any pollen of inappropriate morphology or size was not counted. The background pollen counts for a given run, estimated by the average of all upwind samplers, was subtracted from each downwind sampler reading before further analysis.

The total number of pollen grains in the plume emanating from the plot during the sampling period (15 or 30 min) could be estimated for those runs in which the sampling array intercepted all or most of the plume, as evidenced by low or zero pollen numbers on samplers at the lateral and upper margins of the array. The estimate of pollen grains in the plume was made by first calculating the aerial concentration of pollen measured by each sampler. Concentration was calculated as the number of pollen grains on both rods divided by the volume of air sampled (circumference of the path of rod movement × collection rods' surface area × number of revolutions during the sampling). Pollen counts were also corrected for the collection efficiency of the sampler, 60%. The theoretical efficiency was calculated (Aylor, 1993), from the pollen settling velocity and the collector size and speed, to be 86%. This value was adjusted downward to 60% based on a report (Ogden and Raynor, 1967) that ragweed pollen is collected by rotary impaction samplers at about 65 to 70% of the theoretical efficiency. Although there is no comparable information on actual vs. theoretical collection efficiency for grass pollen, the ragweed pollen is of similar size (20 μm diam.) to that of bentgrass and should therefore have similar impaction properties. The pollen flux through the sampling array was calculated by using a simple numerical integration of the observed concentrations and wind speeds, as follows. The area represented by each sampler in the array was assumed to extend half the distance to the next sampler, both horizontally and vertically (Fig. 1). The pollen flux for each sampler was calculated as pollen concentration (pollen grains/m^3) × volume of air (m^3) moving past the sampler during the sample period. This air volume was calculated as the cross-sectional area of the conceptual rectangle surrounding the sampler (Fig. 1) multiplied by the wind run during the sampling period (wind speed in m/s × total seconds). Average wind speed during the sampling period for each sampler height was obtained by logarithmic interpolation (Thom, 1975) of wind speed measurements at 0.5, 2.0, and 6.7 m height. Total flux of pollen through the cross-section of the pollen plume was obtained by summing the fluxes from all of the samplers.

Weather data at the pollen sampling site were collected at 5-min intervals with automated weather instrumentation (Campbell Scientific Instruments, Logan, UT). Wind speed was measured with rotating cup anemometers at 0.5, 2.0, and 6.7 m above ground level, and wind direction was measured at 6.7 m height. Air temperature was measured at 0.5, 1.5, and 6.5 m height. Sensors for rainfall, total solar radiation and relative humidity were placed at 4.6, 3.9, and 1.5 m height, respectively. Measurements for computing turbulence parameters (standard deviation of the vertical and horizontal wind speed) were obtained with a sonic anemometer (model CSAT3, Campbell Scientific Instruments, Logan, UT) mounted at 1.7 m above ground level and facing into the wind. The sonic anemometer was operated at 1 Hz, and was located in the oats approximately 40 m away from the creeping bentgrass plot, 45 to 90° from the downwind direction.

The diurnal pattern of pollen release was measured with a Burkhard 7-d recording suction sampler at 2-h resolution and operating at a height of 0.75 m. It was not desirable to place the sampler in the 6-m circular plot where the plume was sampled, because of the effect such a large object would have on turbulence and thus on the pollen release being measured. Therefore the sampler was placed within a commercial field of creeping bentgrass (Seaside) approximately 2 km from the plume sampling site. Pollen counts from this sampler were used to construct a temporal profile of pollen release on each day, which was taken to represent the diurnal pattern at the plume sampling site on the same day.

Running the CALPUFF Model

The procedure and model settings used in running CALPUFF were as described previously (Pfender et al., 2006). As a brief description, a 420 by 445 km modeling domain approximately conterminous with the state borders of Oregon was created. The MM5 output for each day was processed with CALMET to account for terrain effects and to format the data for use by CALPUFF. CALPUFF was run with a 1-h time step for a 24-h duration for each model run. There were no precipitation events during the days we modeled, so only the dry deposition (not the wet deposition) module of CALPUFF was used. We modified the deposition reference height in CALPUFF, from 10 to 0.5 m, to better capture the near-source concentration of the pollen, which is released at canopy height. Inputs were used for the average and the variance of pollen settling velocity.

CALPUFF requires an input of area-source emission rate, or pollen grains emitted per unit area of the field per unit time. CALPUFF can incorporate a different emission rate for each 1-h modeled time step. We used data from the plume-sampling plot to estimate emission rate during the sampling period, then assigned emission rates to each hour of the day according to results from the Burkhard sampler. For the sampling-period emission rate, we first estimated the total flux of pollen through the sampling array as described in the previous section. Using an iterative procedure, we then found an emission rate in CALPUFF that would produce the observed mass flux at the sampling array located 10 m downwind of the plot center. This approach matched the simulated emission rate to the observed pollen flux, without the need to consider quantifying pollen production, escape fraction or near-source (<10 m) deposition. In this way, dispersal and deposition could be modeled beyond the 10-m sampling boundary. The input for hourly emission rate was varied over the 24-h simulation run by reference to the diurnal pattern of pollen release obtained from the Burkhard sampler for each respective day's simulation. The daily total pollen flux was determined as the quotient of observed 15- or 30-min pollen flux divided by the Burkard-derived proportion of the daily pollen total that occurred during that time interval. The emission rate for each hour was adjusted as a proportion of the total according to the Burkhard sampling results. For simulations from an area source, CALPUFF uses a 2-dimensional integration algorithm (cross-wind and along-wind) (Scire et al., 2000) that avoids the errors within and near the area source that would be obtained if the total emission were assigned to a single virtual point in the center of the field.

The time-varying emission rates and the observed and modeled weather conditions were used to conduct model runs for several scenarios. We simulated dispersal for two specific dates, selected to span a range of weather conditions in favorability for dispersal. To select dates for the simulations, we first ran CALPUFF for the 15 consecutive days between our first and last plume sampling dates (25 June and 8 July) with a standardized daily emission rate profile, so that deposition results for different days would reflect differences only in weather conditions, not emission rates. The sum of simulated pollen deposition at a ring 3 km from the plot center was evaluated for each day, and the day with the lowest and highest deposition sums were selected. For each of these two dates, two source field sizes were selected for the simulations (2.4 and 25 ha) to match the 10th and 90th percentile sizes of creeping bentgrass seed production fields in Oregon. We also modeled dispersal and deposition of pollen occurring from a "dust devil", a type of vortex thermal updraft (Hess and Spillane, 1990; Sinclair, 1969) that occurs commonly in the grass seed-producing region in summer.

For each simulation scenario, CALPUFF model outputs were obtained for mass balance, with compartments for pollen emitted, deposited on the surface, and airborne in or above the mixed boundary layer. Outputs were produced also for spatially explicit (gridded) deposition to the surface, allowing us to map deposition isopleths in units of pollen grains per m^2. Pollen survival dynamics were incorporated into the model by estimating travel time to each deposition location. The surface distance from the source center to each deposition grid point was calculated, and divided by mean wind speed (6.7 m height) recorded during each 1-h time step to produce the estimate of time required for the pollen grains to reach that location. A negative exponential equation for pollen survival as a function of time, derived from our experiments, was then used to calculate the fraction of the pollen still viable when it reached that location. Survival fractions thus obtained were applied to estimates of pollen deposition at each grid point to produce the gridded data of the viable pollen for final mapping.

RESULTS

Pollen Characteristics and Survival Dynamics

The average diameter for pollen collected from creeping bentgrass cultivar Seaside was 25.4 μm, and average weight was 5.2 ng per pollen grain. The settling velocity for creeping bentgrass pollen grains in still air, averaged across four experiments, was 1.9 cm/s with a variance of 0.28. In eight experiments on survival of creeping bentgrass pollen exposed to outdoor conditions, average viability (germination on a defined medium) decreased from 60% at the time of pollen release to 1% 120 min later and to 0% by 180 min after release (Fig. 2). The survival data were fit well with the equation: % surviving = 100 × [$0.6e^{(-0.036 \times \min)}$].

Observed Pollen Emission Rates and Diurnal Patterns

On 7 d between 25 June 2005 and 8 July 2005, a total of 11 experiments were conducted (Table 1). The observed pollen flux through the sampling array ranged from 2.6 × 10^6 to 7.5 × 10^7 pollen grains per min. Modeled emission rates to produce these fluxes ranged from 2.9 × 10^5

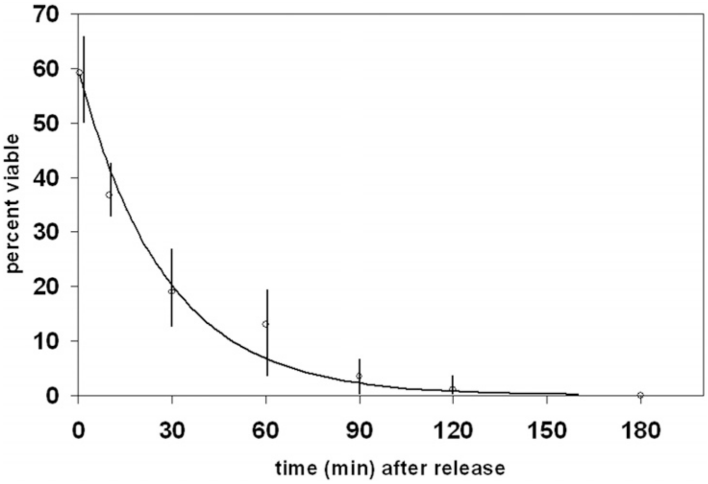

Figure 2. Survival of creeping bentgrass pollen exposed outdoors. Pollen, collected within 15 min of anther dehiscence, was immobilized on traps made of feather down. Viability was assessed as germinability on an artificial medium. Each data point is the average of seven experiments, three replicate traps per time interval per experiment. Vertical bars represent standard error of the mean. The smooth curve is best-fit exponential decline equation: Proportion surviving = $0.6e^{(-0.036 \times \text{min})}$.

to 8.3×10^6 pollen grains emitted per min per m^2 of the creeping bentgrass stand. Conditions of temperature, relative humidity, and windspeed are provided in Table 1.

The diurnal patterns for pollen concentration in the air 0.75 m above a commercial creeping bentgrass seed-production field, on the same days as the nearby plume sampling experiments were done, are shown in Fig. 3. Peak concentration occurred in the 2-h period between 1000 and 1200 h on each day. On some days there was a much smaller, secondary peak sometime between 1500 and 1900 h. Peak concentrations among the sampled days ranged from approximately 9,000 pollen grains/m^3 (8 July) to 30,000 pollen grains/m^3 (25 June and 1 July).

Modeled Dispersal and Deposition

For the modeling effort, we selected 2 d to exemplify the least and most conducive weather with respect to pollen dispersal. To compare 14 d (25 June–8 July) with respect to effects of weather on dispersal, without the confounding factor of variable emission rates, we applied a standard pollen emission rate and diurnal emission profile to all days and compared CALPUFF-modeled deposition among them. To avoid the effects of wind direction on the comparison, we summed modeled deposition in a complete ring (360 degrees, one receptor per degree) at 3 km from the center the plot. There was a range of greater than three orders of magnitude in this modeled deposition among the days, due to the differences in weather conditions simulated to affect the processes (Fig. 4). Among the days for which we had sampled the emission plume (Table 1), 25 June and 8 July were selected to represent days with weather less and more favorable to dispersal, respectively.

On 25 June the plume emanating from the 6 by 6 m test plot was well centered on the sampling array (Fig. 5A). The largest recorded concentration of pollen grains was located at 0.5 m height in the middle of the array, with successively lower concentrations to the sides and at greater height. The tallest sampler (at 4.0 m height) measured a concentration only 2% as great, and the flanking 0.5-m samplers measured <1% as great, as the maximum. Therefore the estimate of pollen emission obtained by the sampling array, 1.5×10^7 pollen grains/min (Table 1), is a good estimate of total pollen flux at 10 m from the plot center. In the CALPUFF simulation for 25 June, this flux could be produced by an

Table 1. Weather conditions, measured pollen flux, and modeled pollen emission rate for field experiments with creeping bentgrass.

Date	Start time	Duration	Temperature at 1.4 m height	Temperature gradient†	Relative humidity at 1.4 m height	Windspeed at 2.5 m height	Windspeed at 6.7 m height	Variance of vertical windspeed‡	Pollen flux through array§	Modeled pollen emission rate¶
	h	min	°C	°C/m	%	—— m/s ——			grains/min	grains/m^2/min
6/25/05	0955	30	17.9	-0.50	71	2.8	3.6	0.28	1.52×10^7	3.68×10^6
7/1/05	1010	30	16.8	-0.62	65	3.0	3.7	nd#	7.48×10^7	8.34×10^6
7/1/05	1105	30	18.6	-0.81	57	3.7	4.3	0.34	4.87×10^7	5.10×10^6
7/3/05	1045	15	19.6	-0.78	56	3.1	3.4	0.39	3.77×10^7	4.20×10^6
7/5/05	1115	15	22.9	-0.79	52	2.7	3.2	nd	2.24×10^6	2.50×10^5
7/5/05	1200	15	24.2	-0.81	47	2.4	2.8	nd	2.47×10^6	2.70×10^5
7/6/05	1200	15	23.2	-0.79	54	3.5	4.2	0.44	4.30×10^6	4.70×10^5
7/6/05	1245	15	23.0	-0.73	50	3.9	4.6	0.51	2.60×10^6	2.91×10^5
7/7/05	1210	15	20.2	-0.67	49	2.5	3.0	0.40	1.08×10^7	1.20×10^6
7/8/05	1130	15	19.4	-0.29	60	2.9	3.5	0.25	6.95×10^6	7.74×10^5

†Change in temperature per meter change in height, from 0.5 to 6.5 m aboveground.

‡Determined from measurements taken with a sonic anemometer.

§Calculated number of pollen grains per min passing through the sampling array located 10 m downwind of a 6- × 6-m plot of creeping bentgrass.

¶Number of pollen grains emitted per min per m^2 of the creeping bentgrass plot, as determined by iterative modeling with CALPUF to simulate the calculated pollen flux.

#nd = not determined.

emission rate of 3.7×10^6 pollen grains per m^2 field area per min. This emission rate was used in CALPUFF simulations of pollen dispersal and deposition for two field sizes (2.4 and 25 ha), using 25 June data for weather inputs and diurnal emission pattern. For the 2.4-ha field, this emission rate produced a total peak hourly emission of 5×10^{12} pollen grains, and a total day's emission of 2×10^{13} grains. CALPUFF output from the 2.4-ha source field (Fig. 5B) shows deposition of 100 viable pollen grains per m^2 reaching 3.2 km, and deposition of one viable pollen grain per 10 m^2 reaching 4.2 km, to the south of the field. In the simulation using the larger source field (25 ha) for 25 June, deposition of 100 pollen grains per m^2 reached 3.3 km, and deposition of one pollen grain per 10 m^2 reached 5 km, from the source field (Fig. 5C).

The sampling array intercepted most of the pollen plume emanating from the 6 by 6 m plot on 8 July, also (Fig. 6A). The emission rate as estimated in the CALPUFF simulation was 7.7×10^5 pollen grains per min per m^2 of plot area. For a simulated 2.4-ha field, the modeled emission rate produce a total peak hourly emission of 1×10^{12} pollen grains, and a total daily emission of 3×10^{12} grains. The CALPUFF simulation for this 2.4-ha field with 8 July weather produced deposition of 100 viable pollen grains per m^2 at a distance of 5.9 km southeast of the field, and one viable pollen grain per 10 m^2 at 6.4 km from the field (Fig. 6B). From the 25-ha field simulation, the distance to these same deposition levels was 6.4 and 6.7 km, respectively (Fig. 6C).

For the simulation of pollen dispersed by a dust devil vortex we assumed a 5-m diam. vortex moving 30 m before dissipating, based on our observations of typical dust devil size and travel distance in the grass seed production region. We further assumed that the vortex would pick up 1 h of pollen emission, and for simplicity assumed that 25% of the entrained pollen was carried to each of the following heights before the vortex dissipated: 5, 10, 15, and 20 m. In this scenario, simulated to occur at the time of peak pollen release on 8 July, CALPUFF produced deposition of 100 viable pollen grains per m^2 at a distance of 5 km from the source, and deposition of one viable pollen grain per 10 m^2 at 15.3 km from the source (Fig. 7).

Results from the five simulations are compared in Fig. 8. The influence of weather can be seen in the dispersal distances being nearly doubled for many deposition levels on 8 July compared to 25 June. For each date, the larger source field produced pollen deposition isopleths at slightly greater distances than the smaller field, but source size was generally less influential than weather as a factor except at the highest deposition level. The importance of conditions that can lift pollen to even a moderate height at the source can be seen in the outcome for the dust devil scenario, where deposition of 1 or 0.1 viable pollen grains per m^2 extends 1.5 or 2 times as far, respectively, as in the nonvortex simulations for the same weather context.

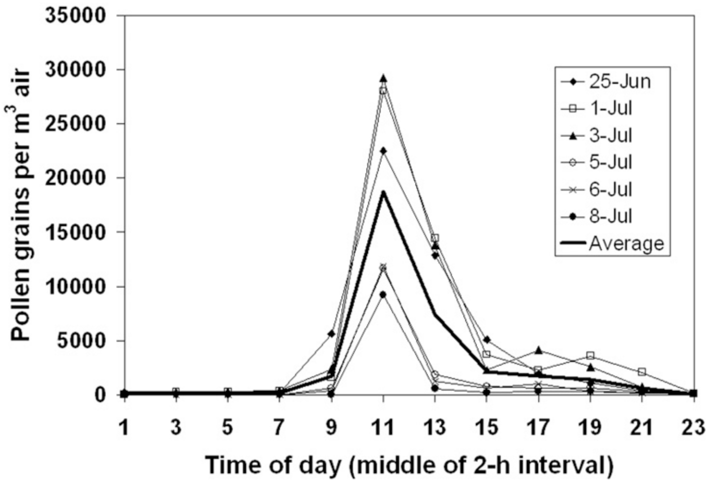

Figure 3. Diurnal patterns of creeping bentgrass pollen in the air 0.75 m above a commercial creeping bentgrass field, located approximately 2 km from pollen plume sampling site, for several days during anthesis. Data were collected with a Burkhard 7-d volumetric suction sampler, read as pollen grains per air volume per 2-h time interval.

DISCUSSION

Results for survival dynamics of creeping bentgrass pollen confirm observations of other researchers that grass pollen does not survive more than 2 h after shedding. However, we observed a more rapid decline in viability during the first hour than was observed by Fei and Nelson (2003), perhaps because we exposed pollen to outdoor conditions whereas

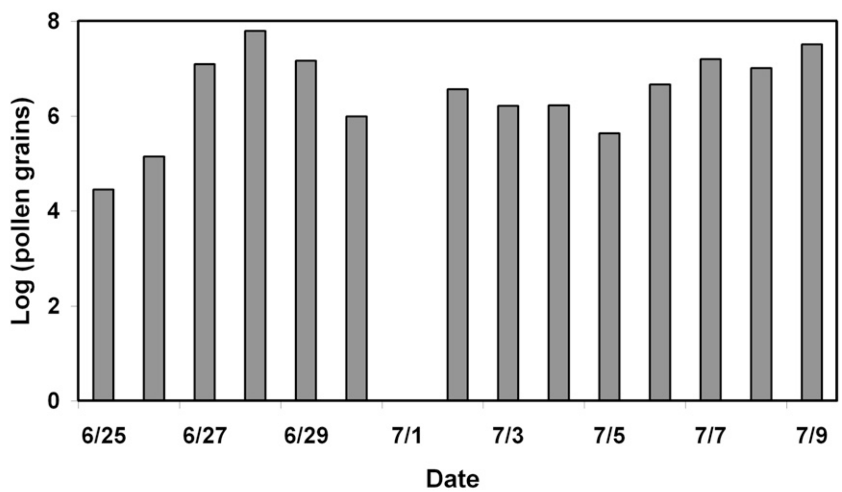

Figure 4. Comparison of days for favorability to pollen dispersal. A standard diurnal pattern of pollen emission was applied to actual weather conditions on each of the days between 25 June and 9 July 2005. Simulations of dispersal and deposition were run in CALPUFF. The height of each bar is the sum of the number of pollen grains modeled to be deposited at 360 receptor sites located in a ring (one receptor per degree) with a radius of 3 km from the test plot. Complete weather data for 1 July were not available.

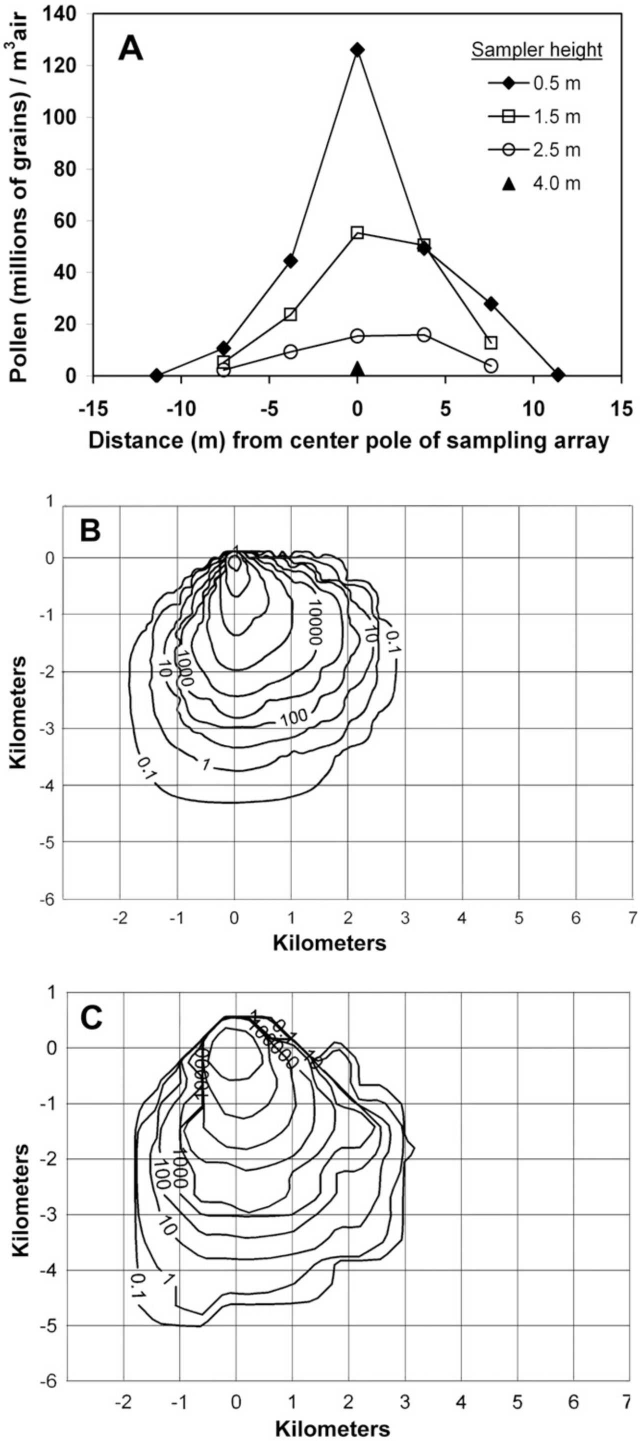

Figure 5. Sampling results and modeled deposition for creeping bentgrass pollen on 25 June 2005. (A) Pollen concentrations measured by rotary impaction samplers in the sampling array depicted in Fig. 1. Background pollen concentrations, measured upwind of the plot, have been subtracted. (B) CALPUFF-simulated dry deposition of pollen grains from a 2.4-ha field of creeping bentgrass, using the diurnal pattern of pollen emission rates and the weather conditions that occurred on 25 June 2005. Isopleth units are the number of viable pollen grains deposited per m^2 of surface area during 24 h (2400–2400 h). (C) CALPUFF-simulated dry deposition from a 25-ha field on the same date.

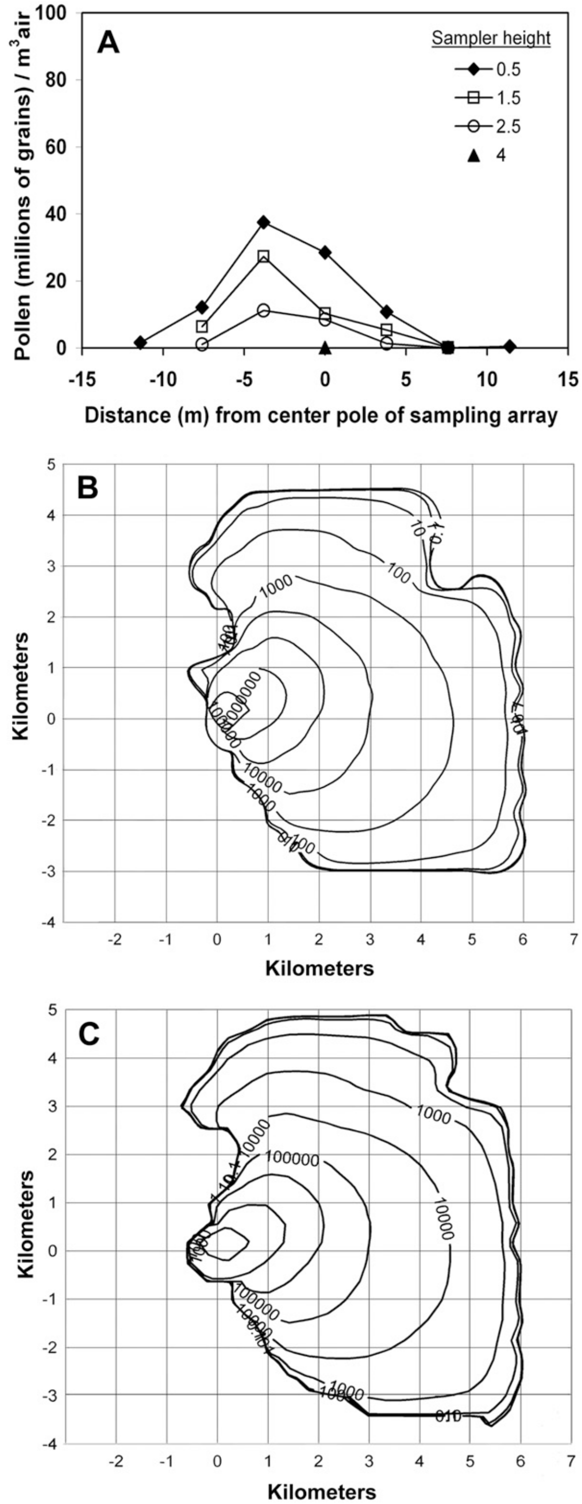

Figure 6. Sampling results and modeled deposition for creeping bentgrass pollen on 8 July 2005. (A) Pollen concentrations measured by rotary impaction samplers in the sampling array depicted in Fig. 1. Background pollen concentrations, measured upwind of the plot, have been subtracted. (B) CALPUFF-simulated dry deposition of pollen grains from a 2.4-ha field of creeping bentgrass, using the diurnal pattern of pollen emission rates and the weather conditions that occurred on 8 July 2005. Isopleths units are number of viable pollen grains deposited per m^2 of surface area during 24 h (2400–2400 h). (C) CALPUFF-simulated dry deposition from a 25-ha field on the same date.

they stored pollen in a desiccator during the survival test. In any case, it is clear that the limited duration of pollen viability must be considered in evaluating the potential for pollen-mediated gene flow. Data indicate that an exponential decline is a good description of survival dynamics for creeping bentgrass. The diurnal shedding pattern we observed for creeping bentgrass pollen was similar to that reported previously (Fei and Nelson, 2003), in that peak shedding occurred in the morning. However, whereas Fei and Nelson (2003) reported the peak to occur at 0900 h with a major second peak at 1400 h, we observed the peak to occur between 1000 h and 1200 h with a very small or non-existent afternoon peak. These differences could be due to cultivar or environmental factors, since Fei and Nelson's (2003) data are from cultivar Crenshaw growing under greenhouse conditions with supplemental lighting.

We measured pollen flux through a sampling array to estimate emission rate of pollen from a creeping bentgrass field. Pollen emission rate for a grass stand has not previously been reported, and may be useful in other analyses of pollen movement. Our estimates show that peak emissions can be as high as eight million pollen grains per min from each m^2 of the stand. Calculations using the peak emission rate and diurnal pattern of pollen release show that a 25-ha field of creeping bentgrass can release 10^{14} pollen grains in a day.

Other researchers have noted that movement of wind-blown grass pollen is complex, and difficult to describe adequately with simple dispersal models (Giddings et al., 1997b; Jackson and Lyford, 1999; Nurminiemi et al., 1998; Rognli et al., 2000). CALPUFF accounts for complexities in air-borne pollen movement caused by atmospheric instability, changing conditions of wind speed and direction and effects of terrain. Furthermore it allows for a time-varying release rate (as seen in Fig. 3), and for matching these rates with the appropriate time-varying weather conditions during the course of a day. Therefore, this modeling tool should be useful to estimate pollen movement across a range of situations. Given the complexity of particle dispersal in the atmosphere, however, it is nonetheless unrealistic to expect highly accurate determination of pollen deposition. In validation studies CALPUFF has provided estimates of mean concentrations that may differ from actual values by a factor of two or more (Chang et al., 2003), although estimates of maximum concentrations may be much more accurate (Hurley and Luhar, 2005).

It was not possible to directly validate the modeling results for pollen dispersal and deposition in our study, given the great dilution of pollen concentrations with distance and the multiplicity of other pollen sources whose emissions would overlap beyond several kilometers away from the source. Indeed, it is precisely because of the impossibility of direct observation that modeling is useful. CALPUFF has been extensively tested and validated previously for wind-dispersed particles (Scire et al., 1990). Because we demonstrated and quantified the pollen flux in the airborne plume, we can be confident that the model based on the computed emission

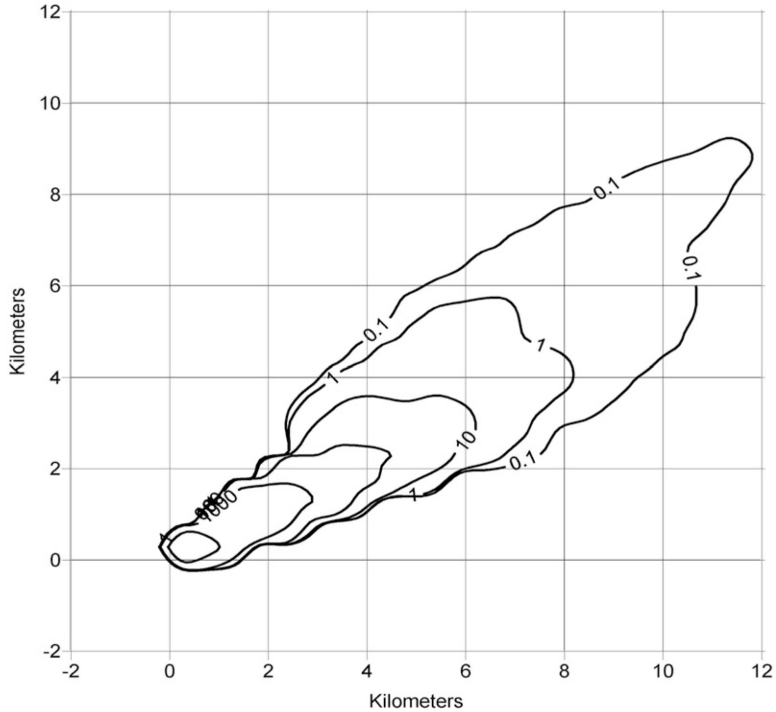

Figure 7. CALPUFF modeling results for a simulation in which creeping bentgrass pollen was lifted as much as 20 m above the ground in a single event by a hypothetical 5-m diam. thermal vortex ("dust devil") occurring during weather conditions that existed on 8 July 2005. Map shows deposition in units of viable pollen grains per m^2 surface area.

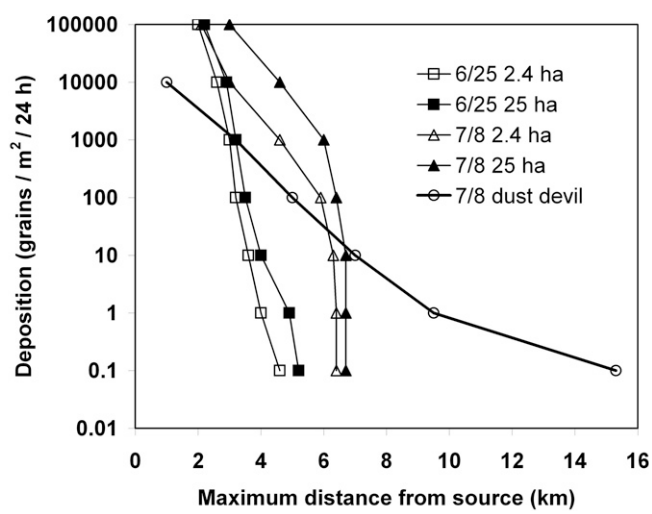

Figure 8. Comparison of modeled pollen deposition for two dates differing in weather conditions and pollen emission profiles, for two field sizes representing the 10th and 90th percentile sizes of creeping bentgrass seed production fields in Oregon (2.4 and 25 ha). For one of the days, simulation results from a hypothetical dust devil event are also shown. Values were derived from maps shown in Fig. 5 to 7, and represent the maximum distances for each of the indicated viable pollen deposition concentrations.

rates provides realistic results for dispersal and deposition of these particles. As noted previously, precise values for deposition are not likely to be obtainable by modeling, but the features of extent and maximum concentrations should be well approximated by this approach. The general features of the modeled pollen deposition are congruent with other reports, particularly the observations of gene flow in the vicinity of genetically modified creeping bentgrass in central Oregon (Watrud et al., 2004). For example, the CALPUFF output shows high pollen concentrations deposited within several kilometers of the source field, and deposition over a wide area (e.g., from north to southeast of the source field for the 8 July simulation; Fig. 6C) due to variable wind direction. Some conditions are such that a substantial density of pollen deposition is expected also upwind of the prevailing wind direction, for example at distances up to 0.1 km west of the edge of the source field on 8 July (Fig. 6C). Watrud et al. (2004) observed a predominance of gene transfer within several kilometers of the source, including to nearby locations upwind of the prevailing wind direction.

Particularly instructive are the CALPUFF results from a scenario in which a thermal vortex (dust devil) aids pollen movement (Fig. 7). Dust devils are common in warm, sunny areas (Hess and Spillane, 1990; Sinclair, 1969) such as occur in the seed-producing regions of western and central Oregon during June and July, the months when creeping bentgrass is flowering,. It seems likely that thermal vortexes, which can readily move pollen more than 10 km from the source, were involved in gene flow 14 to 21 km from the source in the central Oregon case (Watrud et al., 2004). The site of the central Oregon observations, high desert on a plateau near steep canyons (Watrud et al., 2004; Van de Water et al, 2007), would be favorable to development of turbulence including thermal vortexes. In our CALPUFF simulation, greater windspeed or less conservative assumptions about the height of the vortex and the amount of pollen entrained would have resulted in modeled deposition at somewhat greater distances than shown in Fig. 7. A calculation based simply on survival time and average windspeed may overestimate the distance for dispersal, however, because the pollen must be deposited from the air column to be effective in pollination. After reaching appreciable height, probability of immediate deposition decreases due to the relatively slow settling velocity of pollen. Our CALPUFF results indicated that pollen lifted by a dust devil would travel hundreds of kilometers from the source (results not shown), but their 3-h survival limit should render these travel distances inconsequential for gene flow.

Field source size was found to have a distinct but rather limited effect on extent of pollen deposition, affecting mostly the deposition density near the source field. The weather conditions during dispersal, on the other hand, were more influential in the modeled outcome. For example, under the assumption of an identical daily pollen emission pattern imposed across several days with unique weather, large differences in pollen deposition 3 km from the source were produced among the days (Fig. 4). Also, to the extent that terrain has a significant effect on turbulence and windflow, we can expect different pollen deposition patterns in geographically differing areas.

The relationship of pollen deposition concentration to effective pollination is not directly predictable, because probability of gene flow depends on factors in addition to pollen deposition density. Fertilization probability can be significantly higher for isolated target plants than for plants growing in a group where local pollen could overwhelm incoming pollen (Rognli et al., 2000). Therefore, we cannot provide an estimate of pollination probability for the mapped pollen isopleths in Fig. 5 to 7. However, it is clear that large pollen concentrations can occur several kilometers from the source. Our simulation for 8 July shows 100,000 viable pollen grains per m^2 deposited at a distance of 2 to 3 km from the source, and 10,000 viable grains per m^2 at 4.6 km from the source. Thus it seems likely that pollination can occur at these distances. Fertilization from lower pollen deposition densities (e.g., one viable pollen grain per m^2) has a low, but nonzero, probability. Estimates of low pollen deposition densities at distances of 10 to15 km (for the vortex-aided dispersal) support the findings of Watrud et al. (2004), that pollination could occur at these distances.

By using a model based on physical principles (e.g., wind turbulence and pollen settling velocity), the results of this research demonstrate the wide area over which pollen can be deposited from a source field during even a single day, and provide a method to estimate pollen movement under any given conditions during a growing season. This wide distribution, combined with the very large pollen emission rates we measured, suggest it would be exceedingly unlikely to achieve genetic isolation for a field planting of a creeping bentgrass crop unless inter-field distances of at least several kilometers were maintained. At closer distances, pollen competition at the receiving field could reduce gene transfer to a low level, and this level of transfer may be acceptable for routine considerations such as cultivar purity. But if a zero-tolerance criterion is used, as may be the case for transgenes in some situations, even low-probability events demonstrated by this research to be possible at distances up to 15 km must be considered.

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